

THE FATE OF *BOTRYLLUS* (ASCIDIACEA) LARVAE COSETTLED WITH PARENTAL COLONIES: BENEFICIAL OR DELETERIOUS CONSEQUENCES?

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ABSTRACT

The consequences of settlement of *Botryllus* larvae close to or on parental colonies were followed in two sets of experiments. In the first, 28 experimental progeny settled adjacent to 6 parents; 207 other sibling progeny served as controls. Four different types of interactions between parent colony and offspring were observed: fusion and resorption of the offspring, fusion and separation, tunic-to-tunic contact and separation, tunic-to-tunic contact, and the death of offspring. Offspring interacting with parents had significantly higher mortality than control offspring. Resorption was the fastest process (one week on average); the two "separation" processes lasted approximately two months. Twenty of the 21 progeny that died after interacting with parents did not grow at all (even after 75 days). All 7 offspring that separated from their parents grew. In two cases of fusion between offspring and adults, large eggs were found within the progeny zooids. Presumably the eggs translocated from the maternal colony through the connecting blood vessels. Only five progeny survived in this set of experiments, a phenomenon which coincided with the degeneration or the mortality of the parent. In the second set of experiments all 93 progeny which had settled on old, dead tunics of 5 parental colonies died within 8 weeks.

These results indicate that cosettlement of offspring proximal to their parental colony is usually deleterious in the long term to the progeny, both when they fuse with or when they merely contact the parent. This phenomenon was also recorded in field observations. We suggest that the phenomenon of gregarious settlement of *Botryllus* larvae near their parents, although characterized by the loss of many progeny, is nonetheless advantageous in response to biotic interactions such as interspecific competition. In this view resorption may have evolved as a secondary process, as a result of the nature of self/nonself recognition in *Botryllus*.

INTRODUCTION

Colonies of the tunicate *Botryllus* originate from a sexually produced planktonic larva. The larva attaches to a substrate and there metamorphoses into a "founder" oozoid. Colonies arise by asexual multiplication (budding) from this "founder" oozoid. The result is a colony of morphologically and genetically identical zooids. The star-shaped groups of zooids in the colony all share a common blood-vascular system embedded in a continuous gelatinous matrix, the tunic. Studies of colony specificity show self/nonself discrimination in the genus *Botryllus* (reviewed by Taneda *et al.*, 1985). When the growing parts of two adjacent colonies of the same species come

into direct contact, they either reject one another or fuse. This histocompatibility (or fusibility) discrimination is controlled by a single gene locus with multiple codominantly expressed alleles (Oka and Watanabe, 1960; Sabbadin, 1962; Scofield *et al.*, 1982; Scofield and Nagashima, 1983). Consequently, two colonies sharing one or both alleles at the fusibility locus can fuse through their vascular blood systems. Colonies that do not share either allele at the fusibility determining locus reject each other.

Grosberg and Quinn (1986) suggested that larvae of *B. schlosseri* from Woods Hole, MA, distinguish kin on the basis of shared fusibility locus alleles, a mechanism which promotes cosettlement of histocompatible colonies. A gregarious settlement of *Botryllus* sibling larvae was also recorded from Monterey, CA (Scofield *et al.*, 1982), and in populations from the Mediterranean Sea (Sabbadin, 1978). Sibling cosettlement in *Botryllus* may be intensified rapidly by the mechanism of larval metamorphosis after larval release into the water column (Grave and Woodbridge, 1924; Sabbadin, 1978; Grosberg and Quinn, 1986). This promotes cosettlement of larvae in proximity to their parental colonies (Sabbadin, 1978; Grosberg and Quinn, 1986; our unpubl. data). Offspring share at least one allele with their parental colony at the fusibility-histocompatibility locus. Therefore they have the capacity to fuse with the parent colony.

Grosberg and Quinn (1986) proposed that colony fusion among kin may be beneficial to both members of the chimera in several ways. For example, colony fusion immediately increases the chimeric-colony size. Since survivorship and onset of reproduction are known to be size dependent, fusion might reduce the likelihood of mortality and lower the age of first reproduction. Here we describe a laboratory study of the consequences of offspring cosettlement near their natal colony. Survivorship and growth rates of the larvae were followed and compared to other, control offspring. In another set of experiments we followed the survivorship of progeny settled on their maternal-colony tunic.

MATERIALS AND METHODS

Eleven large sexually matured colonies of Monterey *Botryllus schlosseri* were used in two sets of experiments. The sexual maturity of each colony was determined by embryos developing inside the zooids. All the large colonies were born and raised in the laboratory in a standing seawater system (Boyd *et al.*, 1986) until their use in experiments. Thereafter they were maintained in a running seawater system (Rinkevich and Weissman, 1987a). Two sets of experiments were performed. In the first, larvae were allowed to settle near their parental colonies. In the second, larvae were allowed to settle on maternal-colony tunic.

First set of experiments

Six colonies (= cases) were placed separately, in six 4 l glass tanks just before larvae were hatched. The tanks were aerated by an airstone and maintained in 18°C by a 50W aquarium heater. The glass slide on which each colony had been grown (5 × 7.5 cm) was placed vertically in a slot of a glass staining rack. A blank slide was placed in the nearest slot, facing the sexually matured colony. Hatched larvae settled immediately after their release on the maternal colony tunic, adjacent to the parental colony, or on the blank slide. Larvae metamorphosed to oozoids rapidly thereafter. The slides were transferred to 17 l tanks in a running seawater system two days later. Progeny that settled on the colony tunic were removed and discarded, as were dying or poorly developed zooids that settled on the two glass slides. Several developed

oozooids (3–7 experimental oozooids in each case) were left undisturbed near their parental colony. Under the dissecting microscope other well-developed oozooids were carefully peeled from the blank slides with small pieces of razor blade attached to a firm handle. They were translocated with a Pasteur pipette to other 5×7.5 cm glass slides to serve as controls (20–44 controls per case, total of 207 control offspring). Observations were taken at least once each week, when the mother-colonies and the offspring were cleaned with a soft small paint brush. Experimental offspring that interacted with the colonies were observed 4–5 times a week. All control offspring of a given colony together with their natal colony and the experimental offspring were kept within the same tank under comparable conditions.

Second set of experiments

Five large, older colonies (ages 15–20 months) were used. Each colony had grown over most of its glass-slide surface area. However, at least 30% of the animal surface area was characterized as “dead tunic.” A dead tunic is designated as the tunic of the resorbed, old part of a large colony that does not encompass ampullae and active blood vessels. A dead tunic quite often also appears between systems which spread apart, in a growth pattern distinct from most colonies found in the wild but which often characterizes laboratory-cultured *Botryllus* colonies. Dead tunic frequently includes relics of resorbed zooids, buds and unhatched embryos, and is covered on its upper side by fouling organisms, and on its lower side (facing the glass substrate) by unicellular algae. In this set of experiments we followed the survivorship of offspring settled on dead tunic of their natal colonies. All other offspring settled on the slide, on the mother colony zooids, or near the big colony were removed by a small piece of razor blade.

RESULTS

First set of experiments

Twenty-eight experimental progeny interacted with the six parental colonies (cases I–VI) in the first set of the experiments. Four different types of interactions were observed and designated as: A = fusion, leading to resorption of the offspring; B = fusion, followed later by separation of the progeny and the maternal colony (disconnection); C = tunic-to-tunic contact between the offspring and the parental colony accompanied by the death of the progeny; D = tunic-to-tunic contact, followed by disconnection of the two interacting individuals (Table I). The colony size of each observed experimental progeny and the mean body size of the control progeny for each case are given in Figures 1 and 2. In this series of experiments (Figs. 1–4, Tables I, II) zooids which remained in contact with the natal colony suffered growth failure and poor survivorship, whereas those zooids which subsequently disconnected from the natal colony both survived and grew. These results are detailed below in a case-by-case analysis.

Case I (Table I, Figs. 1, 3a–d). Seven offspring directly contacted with the parental colony. Five of them (nos. 1, 3, 5, 6, 7; Fig. 1) fused or interacted by tunic-to-tunic contact only and thereafter died or were resorbed without growing. In one case (progeny no. 5) the offspring survived for 76 days, whereas oozooid no. 1 resorbed 48 h after fusion was recorded. When oozooid no. 1's body was resorbed, the left ampullae and blood vessels continued to operate for an additional one month while they gradually disintegrated (Fig. 3a–d). Only two colonies (nos. 2, 4) survived: offspring no. 4 remained attached to the dead tunic of the mother colony for 71 days and then sepa-

TABLE I

Summary of interactions between offspring and their parental colonies in the first set of experiments

Case	No. of offspring	Interaction type ^a				Remarks ^b
		A	B	C	D	
I	7	3	1	2	1	B = Disconnected after 20 days as a result of subsequent degeneration of the mother colony. D = The offspring attached to "dead" tunic of the mother colony.
II	5	—	1	4	—	B = Disconnected after 57 days as a result of subsequent degeneration of the mother colony and the offspring.
III	3	—	1	2	—	B = Disconnected after 81 days as a result of subsequent degeneration of the mother colony and the offspring.
IV	3	—	—	3	—	
V	6	2	1	3	—	B = Disconnected after 61 days as a result of subsequent degeneration of the mother colony.
VI	4	1	1	1	1	B = Disconnected after 63 days as a result of subsequent degeneration of the mother colony. D = The offspring attached to "dead" tunic of the mother colony.
Total:	28	6	5	15	2	

^a The interaction type: A = Fusion and resorption of the offspring; B = Fusion and disconnection; C = Tunic-to-tunic contact and death of the offspring; D = Tunic-to-tunic contact and disconnection.

^b "Dead" tunic = A layer of tunic which attached to the colony but does not harbor zooids, blood vessels, or ampullae.

rated, and no. 2 fused with the degenerating part of the parent for 20 days and then separated. This last colony exhibited a significantly higher growth rate than the controls, and on the final day of the experiment (age of 125 days) it possessed 25 zooids (in 2 systems) compared to 6.8 ± 3.4 zooids of the control offspring ($P < 0.001$, *t*-test).

Case II (Table I, Fig. 1). Five offspring attached to their maternal colony tunic. Four of them (nos. 1, 3, 4, 5; Fig. 1) died. Offspring no. 2 remained fused with the mother colony for 57 days and separated as a result of reciprocal degeneration of the two members of the chimera. This progeny continued to degenerate and died 51 days thereafter. Twelve days after the fusion, when progeny no. 2 possessed only four zooids, we observed two large eggs in its buds. We had followed the interactions between this progeny and the parent every other day from the day of fusion and did not observe any development of female or male gonads in the progeny. Five days later, seven large eggs were recorded in progeny no. 2's buds. After 9 additional days and up to the death of this progeny (at the age of 152 days) no more eggs were recorded. The mother colony contained many eggs during the first 14 days after fusion; these disappeared thereafter concomitantly with its own degeneration.

Case III (Table I, Figs. 1, 3e-h). Three offspring attached to the parent tunic. Two (nos. 2, 3; Fig. 1) died without fusion. Offspring no. 1 fused and disconnected 81 days later as a result of reciprocal degeneration (Figs. 1, 3e-h). This progeny continued to degenerate and died 21 days thereafter.

Case IV (Table I, Fig. 2). Three progeny attached to the mother colony tunic. They died 12-47 days thereafter without growth.

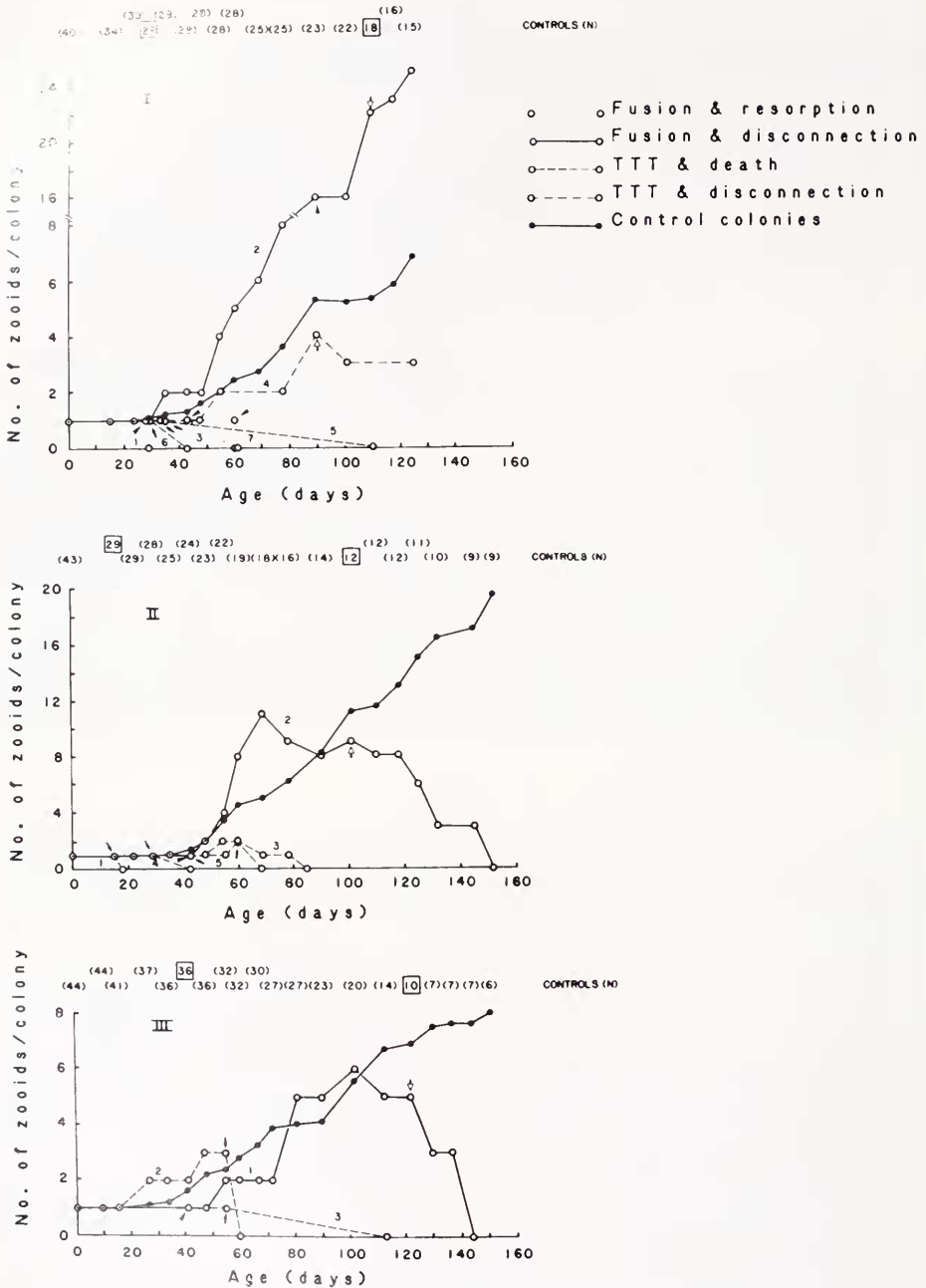


FIGURE 1. *Botryllus lossleri*. Cosettlement of offspring near their maternal colony. Cases I to III, represented by 7, 5, and 3 experimental offspring, respectively (each experimental offspring is marked by a number). The average body size of the controls is marked by a solid line and black circles. The experimental offspring are marked by open circles. A small black circle within an open one represents a situation in which the body sizes of the controls and the experimental offspring are the same. The numbers in parentheses in the upper part of each case represent the number of surviving controls, while those in boxes provide the number of controls on the day of first offspring-parental interaction and on the day of termination of the last offspring-parental interaction. A black arrowhead = fusion; a black arrow with an open head = disconnection; TTT = tunic-to-tunic contact.

No. of zooids/colony

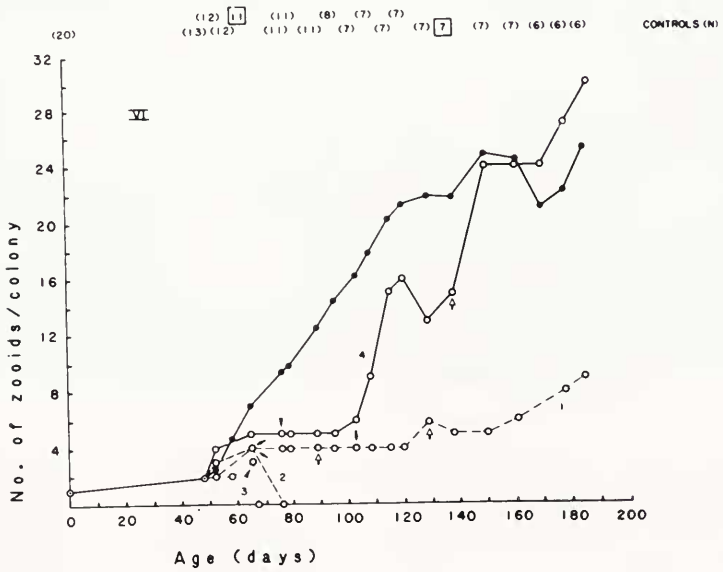
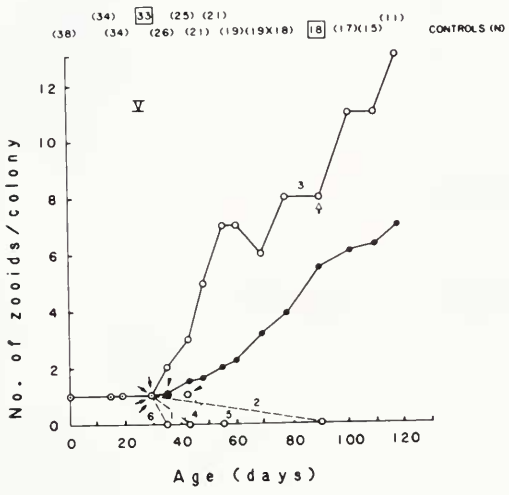
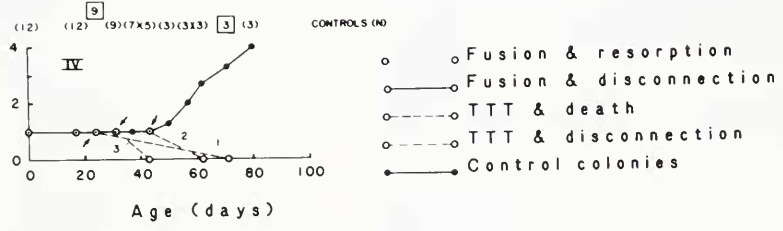


FIGURE 2. *Botryllus schlosseri*. Cosettlement of offspring nearby their maternal colony. Cases IV to VI, represented by 3, 6, and 4 experimental offspring, respectively (each experimental offspring is marked by a number). See legend to Figure 1 for other details.

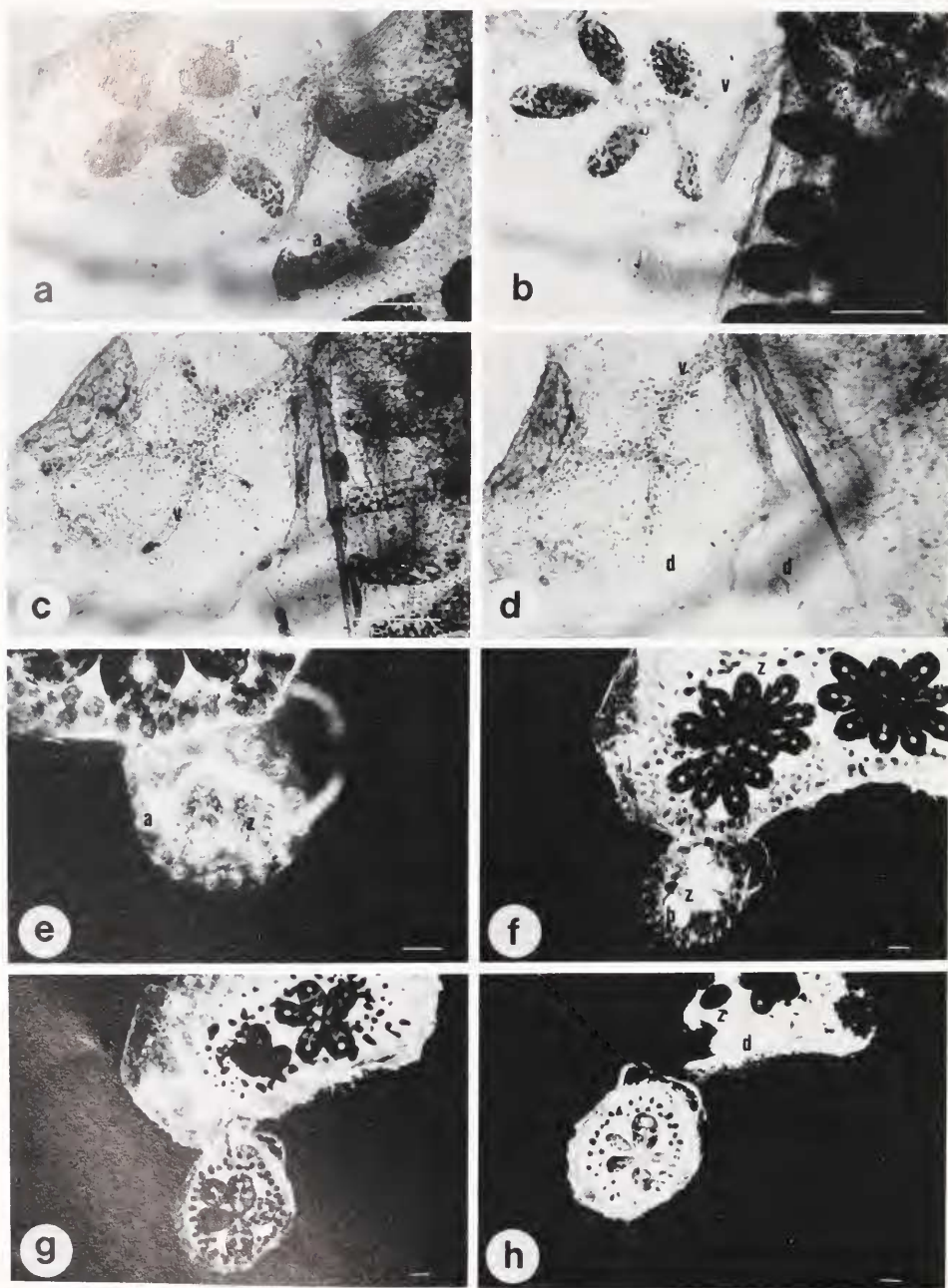


FIGURE 3. Resorption and disconnection of progeny cosettled with their parent. a-d: Case I, offspring no. 1 (refer to Fig. 1). (a) Immediately after resorption, the relict of the resorbed zooid is still seen, with five operating ampullae connected with the parent by one vessel. (b) Six days later, the zooid's body is completely resorbed, while the ampullae are still functional. (c) After an additional eight days, the ampullae are resorbed but the blood vessel still permits blood flow. (d) A month after the zooid's resorption, the blood vessel and ampullae have disintegrated. The dead tunic, overgrown by bacteria, was loosely adherent to the substrate and was detached from the substrate three days later, during a routine cleaning of the

Case V (Table I, Figs. 2, 4a-e). Five of the six offspring that attached tunic-to-tunic or fused with their maternal colony died without growth. A typical fusion-resorption process is documented with offspring no. 4 (Fig. 4a-b). Progeny no. 3 represents another case of egg transfer from the mother colony (Fig. 4c-e). This progeny grew to 2 and 3 zooids 6 and 14 days after fusion, respectively. In this last day (age 43 days) one big egg was observed in a bud (Fig. 4c), but no male gonads were found. The maternal colony contained many similar-size eggs. Progeny no. 3 grew rapidly and 61 days after fusion it disconnected from the parent as a result of degeneration and the death of the mother colony. At the end of the observation period (day 118) progeny no. 3 contained 13 zooids and was significantly larger than the controls (7.0 ± 3.4 zooids; $P < 0.01$, *t*-test).

Case VI (Table I, Fig. 2). Two (nos. 2,3) of the four offspring which attached to the parent colony died or were resorbed within 11 days. Offspring no. 1 attached to the mother colony tunic for 63 days and disconnected because of degeneration of the old part of the parent. This colony grew significantly slower than the controls, and on the day of separation (age 129 days) it contained only 6 zooids compared to 21.9 ± 9.4 zooids of controls ($P < 0.01$; *t*-test). Offspring no. 4 fused with the parent and disconnected after 62 days, concomitant with the large colony's degeneration. On the day of separation (age 138 days) this offspring contained 15 zooids (compared to 21.7 ± 9.5 zooids of controls, $P > 0.05$, *t*-test). Offspring no. 4 multiplied to a 30 zooid colony after an additional period of 47 days.

We calculated the controls' survivorship-percentage in each case by subtracting the number of surviving controls on the day of termination of the last offspring-parental interaction from the number of controls on the day of first offspring-parental interaction, and found 46.3% survivorship (68 out of 147 of the control offspring were alive). By contrast, when we group the offspring which were eliminated by resorption and mortality together, only 7 out of 28 experimental offspring survived (25%). In fact, survivorship eventually was even less: two experimental offspring [case II2 and case III1] were included in the group of survivors although they were already in the process of dying, since according to the pre-determined criterion, they were still alive when re-separated from their parental colonies. The experimental offspring have a significantly higher proportion of "exclusion" than the controls ($P < 0.05$; testing equality of two percentages). However, there is the argument that the resorption of the fused oozoids by their parent-colonies cannot be equated with, and is not equivalent to, the death of those offspring which attached but did not fuse with natal colonies. According to this point of view only 2 out of 17 offspring (11.8%) survived. This mortality rate is significantly higher than that of control offspring ($P < 0.01$; testing equality of two percentages).

animals. e-h: Case III, offspring no. 1 (refer to Fig. 1). (e) Fusion of the offspring with the parent involves one vessel. The offspring possessed two zooids. (f) Seventeen days later, the offspring is a small colony with two zooids and four buds, before changing asexual generations. The ampullae in the parent colony have retreated, and there was no growth of the parent. The nearest two systems to the fused offspring contained 21 zooids. (g) Forty-one days later, degeneration and partial mortality of the parent has occurred, with the two nearest systems to the offspring containing only eight zooids. All ampullae of the parent retreated, exposing a bare, dead tunic. The offspring was still connected by one operating blood vessel to the mother colony. No growth occurred in the offspring colony. (h) Nine days later, resorption and death of most of the maternal colony continued with only four zooids remaining near the offspring. The offspring had disconnected from the mother colony, was in fair condition, but with no growth. a = ampullae, b = bud, d = dead tunic, rz = resorbed zooid, v = blood vessel, z = zooid. Scale bar: a-d = 0.2 mm, e-h = 0.5 mm.

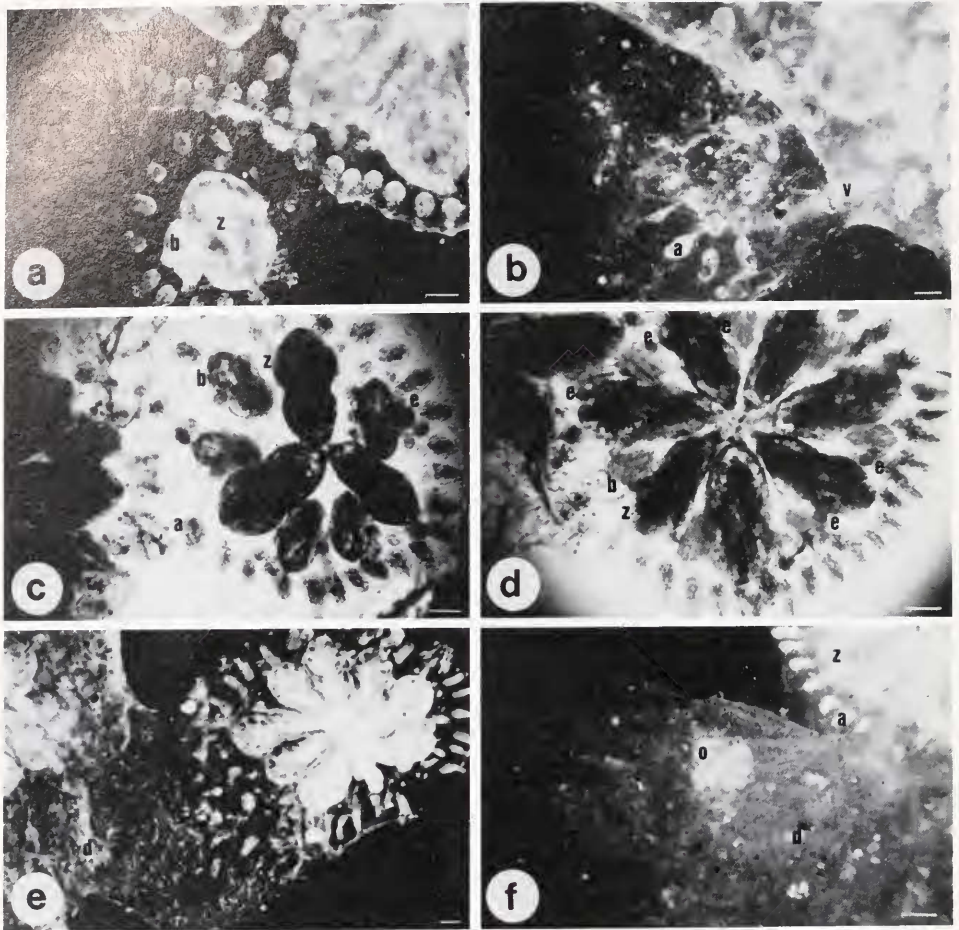


FIGURE 4. Settlement of *Botryllus* larvae near or on the parental colony. a-e: Case V (refer to Fig. 2). a-b: Offspring no. 4, represents a typical resorption. (a) Immediately after fusion. (b) Eight days later, resorption, only ampullae remained. c-e: Offspring no. 3, eggs translocated from the mother colony to the progeny. (c) Fourteen days after fusion, the offspring had three zooids and one egg in a developed bud (picture was taken from underneath the colony). (d) Twelve days later, the offspring colony with seven zooids and seven large eggs (only 5 can easily be recognized) in the developed buds (picture was taken from underneath the colony). (e) Twenty-three days later, shortly before disconnection, the dead tunic was connected between the fused colonies. (f) Second set of experiments. Settlement of oozoid on dead tunic of its maternal colony. a = ampulla, b = bud, d = dead tunic, e = egg, o = oozoid, v = blood vessel, z = zooid. Scale bar = 0.5 mm.

Table II presents some characteristics of the fusion and the tunic-to-tunic interactions between the offspring and parental colonies. The processes which resulted in the elimination of the offspring (resorption and tunic-to-tunic contact followed by a death) lasted a shorter period than the other two processes which resulted in the reparation of the offspring from the parents. Resorption is the fastest process (on the average about one week, Table II) while the two disconnecting processes lasted on average about 2 months. In addition, most of the offspring which ultimately were resorbed and killed during tunic-to-tunic contact did not grow at all (20 out of 21).

By contrast growth was recorded in all the 7 offspring in the other two types of interactions that led to disconnection ($P < 0.01$; Fisher's exact test for independence).

Second set of experiments

The survivorship of 93 offspring settled on the dead tunic of 5 parental colonies was recorded every 2 weeks for a period of 2 months (Table III). The experimental colonies were carefully chosen for their size and the relative large surface area which encompassed dead tunic only (Fig. 4f). Some of the offspring died (natural death, or the effect of the mother colony?). However, most of them were killed by the lifting of the degenerated parental colony tunic from the substrate. Since the offspring were attached to the tunic and not to the substrate, they were swept out from the slide and died. After one month only 18.3% of the offspring survived. Two months after settlement no offspring survived (Table III).

DISCUSSION

Co-settlement of *Botryllus* progeny adjacent to their maternal colony (first set of experiments) or on their mother colony's dead tunic (second set of experiments) clearly resulted in increased offspring mortality. Most of the results in this study were obtained within 2 months of the first tunic-to-tunic contact. Resorption was the fastest interaction recorded (about 1 week) and is much faster than the resorption obtained when two large colonies or two big subclones of different colonies fused (up to 8 months after fusion; Rinkevich and Weissman, 1987a). The fast resorption recorded in the present study could be the result of the huge body-size differences between the parent and the offspring. The colony body size was found to be an important factor for determination as to which colony in a chimera will be resorbed (Rinkevich and Weissman, 1987a). Only 3 out of 11 (27.3%) progeny survived the resorption and the separation from the parent (Figs. 1, 2; Table II). This number is too low to support the proposal that fusion of closely related genotypes on the histocompatibility locus is always beneficial. In fact, the results of the present study indicate that the survivorship of progeny fused with a parent is possible only when associated with the degeneration or mortality of the parental colony (Table I).

Another interesting result in the first set of experiments is that most of the offspring (60.7%) did not fuse with their maternal colonies, although they share alleles in common with the parents in the fusibility locus. Part of this is due to offspring settlement near an "old" part of the parent which did not possess active ampullae. Active, healthy ampullae of growing parts are vital for a successful fusion between compatible colonies (Taneda, 1985; Y. Saito, pers. comm.). However, in other cases, although offspring and parents confronted active, good ampullae, they did not fuse for a long time (up to 2 months); thereafter the offspring died. Out of the 17 tunic-to-tunic interacting pairs only 2 progeny survived: those that reseeded from their parental colony (Table II). In addition, these two offspring (cases I4, VI1; Figs. 1, 2) grew significantly slower than the controls. These results may indicate a possible role of humoral factors and/or cellular elements present in the tunic of intraspecifically interacting *Botryllus* colonies. Diffusing of allogeneic humoral factors through the text matrix was shown to exist in at least two species of botrylloid ascidians, *B. primigenus* and *Botrylloides simodensis* (reviewed by Taneda *et al.*, 1985).

In two cases mature eggs were observed within the offspring soon after fusion with their parents. The parental colonies contained at the same time many similar eggs. In contrast, none of the control progeny exhibited any sign of sexual development.

TABLE II
Characteristics of fusion and tunic-to-tunic contacts between offspring and their maternal colonies in the first set of experiments

Offspring no.	Fusion, resulted in offspring			Tunic-to-tunic, resulted in offspring											
	Resorption			Death											
	Size ^b (zooids)	Time ^c scale (days)	Growth during inter-action	Offspring no.	Size ^b (zooids)	Time ^c scale (days)	Growth during inter-action	Offspring no.	Size ^b (zooids)	Time ^c scale (days)	Growth during inter-action				
I1	1	1	-	I2	16	20	+	I5	1	75	-	I4	1	71	+
I3	1	17	-	II2	1	57	+	I6	1	14	-	VI1	4	63	+
I7	1	1	-	III1	1	81	+	II1	1	3	-				
V4	1	8	-	V3	1	61	+	II3	1	42	+				
V5	1	12	-	VI4	5	62	+	II4	1	15	-				
VI3	3	2	-					II5	2	9	-				
								III2	3	5	-				
								III3	1	58	-				
								IV1	1	47	-				
								IV2	1	18	-				
								IV3	1	12	-				
								V1	1	14	-				
								V2	1	61	-				
								V6	1	6	-				
								VI2	4	11	-				
X ± SD (n)		6.8 ± 6.1 (6)				56.2 ± 19.9 (5)				26.0 ± 22.9 (15)				67.0 ± 4.0 (2)	

^a The offspring numbers refer to the colony case (I to VI); Figures 1 and 2.

^b Size in the day of fusion or colony-to-colony contact.

^c Time scale: the length of the interaction.

TABLE III

Survivship of offspring settled on "dead tunic" of their maternal colonies

Colony no.	No. of settled offspring	No. of offspring after			
		2 weeks	4 weeks	6 weeks	8 weeks
1	25	15	3	0	0
2	18	16	2	0	0
3	12	10	1	0	0
4	22	18	6	2	0
5	16	13	5	1	0
Total	93	72	17	3	0

Egg development in *Botryllus* differs from any other ordinary case of protogyny in that the first developed young ova migrate in the blood stream to the second generation of buds developed from the existing generation of zooids. These ova continue to develop, although they still do not reach maturity. Rather, they migrate again into the next generation of blastozooids. It is not until the seventh or eighth generation that ova are fertilized (Herdman, 1925). Since no such egg development was observed in the fused progeny nor the typical simultaneous male gonad development (Mukai, 1977; Sabbadin and Zaniolo, 1979) the observed large eggs in the fused progeny likely migrated from the mother colony through the connecting blood vessels. Germ cell exchange between fused *Botryllus* colonies has been recorded before (Sabbadin and Zaniolo, 1979). In some hatches of offspring, the progeny released from fused colonies were mostly or totally heterochthonous (Sabbadin, 1982). This suggests the possibility of germ cell parasitism (discussed by Buss, 1982), in addition to the resorption which is related to somatic cell parasitism (Rinkevich and Weissman, 1987a,b). Another pattern of parasitism, oriented translocations of materials from the inferior colony towards the superior member within a chimera, was recorded in fused colonies of the hermatypic coral *Stylophora pistillata* from the Red Sea (Rinkevich and Loya, 1983). This phenomenon was coupled with a significant reduction of growth rate and reproduction of the inferior colony (Rinkevich and Loya, 1985). These findings suggest a possible complex network of physiological interactions occurring after a fusion between two compatible colonies is established. Unfortunately, in these experiments the potential germline chimerism, or parasitism, could not be tested critically, as fertilization of the eggs did not occur. Thus we can neither affirm that the eggs were of parental origin nor that they might have enjoyed a selective reproductive advantage.

Grosberg and Quinn (1986) postulated that *Botryllus schlosseri* larvae recognize kin on the basis of shared alleles at the histocompatibility locus and that this recognition promotes cosettlement of histocompatible individuals. They suggested that fusion which subsequently evolved from cosettlement of the closely related genotypes is beneficial among kin in several ways, as initially proposed by Buss (1982). One of these benefits is that fusion may increase the probability of survivorship and growth. In contrast, the present paper provides evidence (Figs. 1, 2, 3, 4; Tables I, II, III) that the settlement of offspring very near or on their parental colony may in fact reduce survivorship and growth rates. Similar results of reduction in survivorship and growth rates were obtained in other experiments at Hopkins Marine Station in which pairs of large colonies (Rinkevich and Weissman, 1987a,b) or pairs of sibling oozooids (unpub.) were cosettled.

A number of theories have been proposed that the ability to recognize one's kin is beneficial (reviewed by Hepper, 1986). It is a fact that sibling larvae of *Botryllus* settled in aggregations (Grosberg and Quinn, 1986; this paper) and near their parental colony (Sabbadin, 1978; this paper). However, by keeping in mind the deleterious effects of the fusion, how does one explain the existence of cosettlement of closely related individuals such as in the case of *Botryllus*? One possible explanation considers the selective pressure of other biotic interactions, such as interspecific competition.

Botryllus colonies in the field compete for the substrate with other sessile organisms. For example, *B. schlosseri* from Woods Hole is usually overgrown and killed by *Botrylloides* (Grosberg, 1982). In addition, this competition also mediates the relative frequency of different types of life history tactics presented by *Botryllus* colonies (Grosberg, 1982). In Monterey, California, even large *Botryllus* colonies are often overgrown by *Diplosoma*, a fast-growing colonial tunicate (unpub.).

Buss (1981) has shown that the formation of aggregations in the colonial bryozoan *Bugula turrita* is a response to interspecific competition. *B. turrita* also suffers significant intraspecific competition. However, in high densities *B. turrita* is rarely overgrown or killed by interspecific competition (Buss, 1981). The costs incurred by increased intraspecific competition involve a loss of proliferative potential, whereas the benefits accrued by reduced interspecific competition involve whole-colony mortality (Buss, 1981). Since mortality results in a greater loss in fitness than does a reduction in growth rate, the evolutionary selection for the gregarious settlement is plausible. If the persistence of dense assemblages is necessary in sedentary marine animals for excluding possible interspecific competitors (Jackson, 1983), our results could reflect a general pattern of habitat selection in sessile marine invertebrates which aggregate in settlement *versus* those that do not (Knight-Jones and Moyse, 1961; Crisp, 1979; Buss, 1981; Jackson, 1983; and literature therein).

Working on another colonial bryozoan, Keough (1984) demonstrated that larvae of *Bugula neritina* settle preferentially near conspecific larvae. This observation, coupled with the observation that isolated juveniles of this species did not reach adult size, was attributed to the selective effects of predation by fishes. Fishes rarely took more than five bites on any one feeding visit, so groups of colonies might only be damaged at the periphery. A strong advantage to gregariousness resulted since central colonies in a group were left untouched. There is no record of fusion between *Bugula* colonies, although self/nonsel recognition has been described in bryozoans (Keough, 1984).

In *Botryllus*, on the other hand, one common allele at the fusibility locus is sufficient for fusion between colonies (Scofield *et al.*, 1982). Keeping in mind the two studies on gregarious bryozoans (Buss, 1981; Keough, 1984), we suggest that fusion of cosettled compatible *Botryllus* colonies may have long-term deleterious consequences and may not be functionally beneficial to the chimera *per se*, as suggested by Grosberg and Quinn (1986). We propose that the phenomena of resorption and/or death of interacting conspecific colonies may have evolved as a necessary consequence of the self-recognition phenomenon in *Botryllus*, which allows two different genotypes sharing in common only one allele at the fusibility locus to fuse and form a chimera. More specifically, we suggest that the gregarious settlement of *Botryllus* larvae in the proximity of the parental colonies and the nature of self/nonsel recognition of this species may have initially evolved as two independent phenomena (or strategies) which are in the process of adaptation. As a result, the cost/benefit outcomes depend on the view of the observer. The concept for the general costs of fusion was first presented by Buss (1982) and further discussed in Rinkevich and Weissman

(1987b). It is not the purpose of the present paper to discuss again the costs for the chimera in details. However, following our previous discussion, in our view chimera formation in *Botryllus* could develop only if the evolutionary benefits of cosettlement exceeded the disadvantage of the harmful consequences of fusion. Interspecific competition between *Botryllus* and other sessile organisms (Grosberg, 1982) may provide this very selective pressure (Buss, 1981). Gregarious settlement of *Botryllus* larvae, even with the loss of many progeny (as the results of resorption and/or death), would be evolutionarily selected if the number of survivors in gregarious settlement would exceed the number of survivors of settlement in a random distribution. Perhaps the resorption and death of one of the two colonies in parabiotic union allows the unit of reproductive selection to be the survivor of both intra- and interspecific competition. If so, it shall be most important to define the number and character of genes involved in the self/nonsel self recognition events leading to death and/or resorption of contacting *Botryllus* kin. Therefore the ecological advantages of gregarious settlement in *Botryllus* and the role of interspecific competition must be addressed in further field and laboratory experiments.

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